VARIATION IN CORAL MICROBIOME COMPOSITION AND TRANSCRIPTIONAL ACTIVITY OF THREE CORALS OVER DIEL CYCLES

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VARIATION IN CORAL MICROBIOME COMPOSITION AND TRANSCRIPTIONAL ACTIVITY OF THREE CORALS OVER DIEL CYCLES

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ABSTRACT

Understanding the dynamics of coral microbiome composition and function is important because microbiomes plays important roles in coral health and metabolism. While numerous long-term studies have investigated changes in the microbiome due to various physical or biotic stressors, little is known about the stability of the coral microbiome over diel cycle. For certain parameters (e.g., temperature, pH), the magnitude of diel fluctuation can exceed that observed in mean values over seasons, especially on shallow reefs. Such short-term environmental heterogeneity can affect longer term trends, for example by influencing the extent to which corals acclimate to stress and increase resilience. This study examined diel dynamics of microbiomes in three coral species (*Porites lutea, Porites cylindrica, and Pocillopora damicornis)* from a shallow, backreef lagoon in Mo'orea (French Polynesia). *Porites* is relatively resistant to stress, being one of the last coral genera to succumb to bleaching or several diseases, and one of the more abundant genera of corals remaining on degraded reefs. In contrast, *Pocillopora* is less resistant to many of these stresses and bleaches easily but is one of the genera that commonly recruits and rebounds rapidly after disturbances or strong stresses. We assessed microbiome taxonomic composition and relative transcriptional activity by analyzing 16S rRNA gene and transcript sequences from six time points over 48 hours for each of these coral species. Results showed that composition in *P. damicornis* varied significantly over the diel period, while composition in both *Porites* species remained more stable. However, the taxonomic composition of the transcript pool did not vary significantly over time across all corals sampled. This shows that diel stability of these coral microbiomes is dependent on host species, which could contribute to differences in host responses to environmental stressors.

INTRODUCTION

As foundation species, corals are vital to maintaining the function and biodiversity of coral reefs. In order to survive, most reef-building corals rely on the well-characterized symbiosis with the eukaryotic algae *Symbiodinium* to obtain a majority of the coral's required carbon. In addition to this association corals host a range of microbial symbionts, known as a microbiome[1,2]. This microbiome helps to supply the coral with nutrients, such as nitrogen[3,4] and to protect the host from pathogens[5,6]. This latter function is hypothesized to take place through the production of antimicrobial compounds and by outcompeting pathogens for a shared niche[5,6]. Corals form this association by recruiting microbes from the surrounding seawater early in life[7,8] and likely continue to exchange microbes with the seawater throughout life.

The environment around corals is highly dynamic, with fluctuations in physical, chemical, and biological conditions over hourly, daily, and seasonal timescales and also in response to longer term shifts due to climate change. Environmental change is hypothesized to alter the diversity and function of coral-associated microbes. However, despite the microbiome's importance, our knowledge of microbiome change is limited by the timescales over which studies are conducted. For example, while prior work has provided valuable insight into seasonal microbiome fluctuations, only one study has investigated daily (hour to hour) changes in the microbiome[9].

Previous research has shown that many physical and chemical parameters, as well as biological activity, change throughout the day on a coral reef and that these factors affect the organisms living there. Such factors include light levels[10], temperature[11], pH[10,12], oxygen concentration[10], and consumer feeding[13]. Temperature and pH are especially prone to fluctuations on shallow regions of reefs[14]. During the day, when sunlight is available, *Symbiodinium* photosynthesizes and supplies the coral with oxygen for cellular respiration. At night, photosynthesis stops while coral respiration continues, potentially causing the coral tissues to enter a state of hypoxia[10]. The amount of organic carbon (DOC) that corals release also varies with the diel cycle, with levels being highest in the afternoon and dropping off at night[15]. We predict that these daily fluctuations affect the growth, and therefore the taxonomic composition and relative transcription, of the coral microbiome from daytime to nighttime.

To date, few coral microbiome studies have examined daily shifts in composition and metabolic activity, although seasonal changes have been documented[16]. Research has focused on long term problems such as the impacts of disease and climate change. Diel changes have largely been neglected. However, this lack of understanding about short term microbiome shifts raises several issues. Scientists often collect coral samples whenever is convenient due to logistical ease or the difficulties of diving or navigating at night. This leads to inconsistences in the time of day that samples are taken for a study, as well as an underrepresentation of nighttime microbiome samples. Additionally, short term environmental heterogeneity can impact long term responses to stressors[17]. Daily fluctuations in conditions subject an organism to extremes that are often not considered in long term studies on the coral microbiome's response to stress. Currently reefs are exposed to a greater range in pH over the course of a day than is being predicted by mean seasonal variation[12]. Exposures to higher fluctuations in temperature have been shown to increase coral's resistance to bleaching[18,19] and decalcification[20,21], but the potential role of the coral's microbiome in these dynamics is unknown. Characterizing diel shifts in the coral microbiome could provide insights into how the relationship between a coral and its microbiome is affected by daily environmental conditions and convey a need to consider such shifts in long-term studies.

This study aims to understand the diel variation in coral-associated microbiomes, testing the hypothesis that the taxonomic composition and relative transcriptional activity of coral microbiome members fluctuate in response to diel changes in environmental conditions. Corals of three different species, *Porites lutea, Porites cylindrica, and Pocillopora damicornis,* were sampled across six time points in a 48-hour period from a shallow reef surrounding Moorea, French Polynesia. We measured changes in community composition and relative transcriptional activity by sequencing 16S rRNA genes (DNA) and transcripts (RNA), respectively. These data provide some of the first insights into microbial community dynamics and interactions over a diel cycle. Our results have implications for whether daily variation should be incorporated into future studies of microbiome composition and function.

LITERATURE REVIEW

A coral's microbiome is critical for coral nutrient acquisition and health. Several studies have identified genes for nitrogen fixation within the coral microbiome[3,4]. Bioavailable nitrogen is considered a limiting factor on most coral reefs; therefore, microbes that convert nitrogen into forms available to corals may be vital. Coral-associated microbes could also play a role in sulfur cycling[22], hydrocarbon degradation[23], and host protection against pathogens[5,6]. Coral mucus, for example, contains microbes that outcompete pathogens and produce antimicrobial compounds that prevent further colonization by pathogens[5,6].

Despite the important role many microbes perform in corals, the community composition and function can be influenced by variation in environmental factors that can lead to events such as bleaching[24]. Many physical and biotic conditions change throughout a diel cycle; however, only one study looked at how these changes correlated with alterations in the microbiome, specifically focusing on nutrient changes[9] (see below). Due to logistic difficulties, most samples are collected during the day and at variable times. If the microbiome does exhibit daily shifts, this could mean that sampling time is a critical factor in assessing the microbiome and its role in coral ecology. Additionally, as described in Boyd et al. (2016), short term environmental heterogeneity can affect the results of long-term studies. This could be due to environmental variation leading to increased coral resilience to stressors such as coral bleaching and decalcification[18–21,25], possibly through acclimatization to more stressful conditions.

Long term studies have focused on trends in the coral microbiome and how these trends relate to coral health. Most studies have found that there are distinct microbial communities associated with healthy corals versus diseased corals (Reviewed in Mouchka et al. 2010). Increased temperatures create opportunities for pathogens, such as *Vibrio* spp., to infect corals or increase in abundance[27]. This leads to compositional and metabolic shifts in the microbiome. It is currently unknown how quickly corals respond to such stressors in their environment. Thurber et al. (2009) showed that microbiome composition could be shifted to contain more pathogens by a stressor within 64 hours. The generation time for a coastal water column microbial community off Delaware was shown to be 3.6 hours[28]. Most bacteria in the coastal community described grew slower than 0.2 day^{-1}, indicating a small number of taxa brought up the average to 4.6 day^{-1}

showing a wide variety in microbial growth rates with the potential of some members to grow very quickly with growth rates of 21 taxa exceeding 1.0 day^{-1}[28]. In fact, the growth rate of the fastest growing marine bacteria has a growth rate of 140 day^{-1}[28]. Alternatively, environmental stressors may trigger change in the growth or metabolic activity of certain microbes over much shorter time scales. In clownfish gut microbiomes, for example, changes in composition and gene transcription occurred within 1.5 hours of feeding[29] and in the human gut, diet changes can completely restructure the microbiome within a day[30]. It seems reasonable that members in the coral microbiome might have similar capacities for rapid responses to varying conditions.

Many factors such as seawater temperature[11] and pH[12], are considered to be long term stressors of coral reefs. These factors fluctuate on a daily basis[14], and could lead to shifts in the coral microbiome. Cyronak et al (2019) showed that depth was a reliable predictor of how much variation in temperature and pH occurred within a day, with shallow reefs exhibiting the most extreme changes. Temperature and pH generally oscillated throughout the diel cycle[14], with higher temperatures and pH during the day and lower temperature and pH at night[31]. The composition of microbial communities in the water column varies with both temperature and oxygen concentrations[32], among other factors, and several studies have shown that many corals recruit their microbiomes from the surrounding seawater[7,8]. Apprill et al. (2009) showed that coral planulae preferentially recruit certain microbes, but when these taxa are unavailable the coral will incorporate other species into their microbiome. If corals are obtaining microbes from the seawater based on what is available, daily shifts in seawater temperature and pH, may affect composition of the coral microbiome by affecting the composition of the water column microbiome.

Physical and chemical parameters in the coral microenvironment are hypothesized to vary over the diel cycle[10] and to influence microbiome composition and transcription. An extensive study of two coral species demonstrated dramatic differences in tissue oxygen concentration and pH between day and night conditions. During the day, photosynthesis leads to a buildup of oxygen and an increase of pH in the coral tissue. This increase reached a pH of 8.6, which was higher than the pH of the surrounding seawater. However, at night, oxygen levels dropped to less than five percent saturation and pH fell to a low of 7.3[10]. The study also found that these changes, especially in oxygen concentration, occur within minutes of light level increases and decreases[10]. This creates distinct situations in which different microbial groups

may grow optimally and in which others cannot survive. Release of dissolved organic carbon (DOC) displays a diurnal pattern too. DOC release rates at night are less than half of those during the day[15]. Kline et al (2006) showed that differing levels of nutrients also affected the coral microbiome. When dissolved organic carbon levels were high, large amounts of microbial growth could be detected within 26 hours and eventually lead to tissue loss and coral mortality[33]. This indicates that microbes can respond quickly to changes in nutrients and other environmental factors that can have negative impacts on coral health.

Understanding how the coral microbiome changes over short periods (hours to days) is important for understanding the overall effects of environmental variation on corals. A review by Boyd et al. (2016) found that experiments including short term variation in the environment came to different conclusions than studies that did not account for these factors. Such variation in reef conditions exposes corals to extremes that cannot accurately be assessed using mean temperature or pH measurements and predictions. Price et al. (2012) states that reef communities are being exposed to a greater range in pH over the course of a day than is currently being predicted by mean seasonal variation. If the coral microbiome responds to daily fluctuations in environmental conditions similarly to how they respond to long term change, this could have implications for coral health. Understanding daily fluctuations would also inform predictions of how the mean microbiome state may shift under long-term change. One study confirming that daily change is important found significant effects of thermal stress on the coral microbiome only after accounting for daily variation in temperature[34].

Several studies have found that previous exposure to varying conditions can help increase coral resilience to increased temperatures[18,19,25], possibly due to a combination of environmental variation that allows for acclimatization and the presence of heat resistant *Symbiodinium* strains[19,25]. Microbial communities have also been shown to change based on coral heat tolerance[35]. Ziegler et al (2017) found that corals have distinct microbiomes in thermally variable environments and that the microbiome of a coral transplanted to a highly variable environment became similar to the microbiomes of other corals living there. The study also found that the microbiome of corals exposed to variation remained stable when exposed to bleaching conditions, while the microbiome of corals that do not experience variation were changed by bleaching[35]. If coral microbiomes are stable despite environmental variation throughout the day, it could indicate a greater resistance to stressors.

To date, the only study that characterizes daily shifts in coral microbiome composition is by Silveira et al. (2017). This study analyzed microbial communities associated with the coral momentum boundary layer (MBL), which is the second of three layers that exist in the coral mucus and controls the movement of water over the coral[36]. The MBL community represents only a small portion of the coral microbiome and does not include microbes in the rest of the coral mucus, coral tissue, or coral skeleton. The authors predicted changes based on daily variation in environmental factors just as in our study. However, the results showed the MLB community did not change significantly over a diel cycle and was similar to that of the water column, suggesting that the water flowing over the mucus, rather than the coral's physiology, determined microbial composition[9] in the MBL. Looking at microbial communities within host tissues and in the mucus might provide different results that are less influenced by water flow and more influenced by changing environmental or host factors. Moreover, Silveira et al. (2017) examined a single species, *Mussismilia braziliensis,* whose distribution is limited to coastal Brazil. It remains unclear how diel microbiome dynamics vary across host species and in more coral-rich areas such as the tropical Pacific.

The current study assesses how daily shifts in environmental conditions may alter microbial community composition and relative transcriptional activity in three coral species, *Porites lutea, Porites cylindrica,* and *Pocillopora damicornis*. Samples of these species were collected every six hours over a 48-hour period with emphasis on the 1200h and 2400h samples (which were collected on both days, with one 0600h and one 1800h sample to look at trends in the day-night transition periods) in Moorea, French Polynesia. Corals from the genus *Porites* have thicker tissues, which may make them more resistant to thermal stress[37,38], and have been shown to be less effected by bleaching than other genera[39]. Corals from the genus *Pocillopora* appear to be more susceptible to bleaching, but have higher recruitment, growth, and thus population recovery rates than many other corals, like *Acropora* corals[39].

Microbiome composition and transcription were measured by analysis of 16S rRNA genes (DNA) and transcripts (RNA), respectively. By doing so, we gain an understanding of whether or not diel shifts in environmental conditions significantly altered the microbiome. Understanding daily variation gives insights into how quickly the coral microbiome changes and gives researchers a better understanding of how the microbiome and coral may interact biochemically. These results have implications for long term studies that estimate the impact of

ocean change on coral health, potentially suggesting a need to include daily variation in predictions of microbiome change and holobiont resistance to stress. Future studies may need to incorporate daily variance to accurately represent the effects of long-term changes.

MATERIALS AND METHODS

Collection

Small coral fragments (<1 cm^2) were collected from Moorea, French Polynesia on June 2^{nd}-3^{rd}, 2017 at six time points over 48 hours: 2 June (Day1) at 1200h, 1800h, and 2400h; 3 June (Day 2) at 0600h, 1200h, and 2400h. The same five coral heads from each of three coral species (labeled as *Porites lutea* 1-5, *Porites cylindrica* 6-10, *and Pocillopora damicornis* 11-15) were sampled at each time point (Appendix A). All coral heads occurred within the same ~20 m^2 area at a depth of 1-1.5 meters and were chosen to be large enough that multiple samples could be taken (at the six time points) without collecting freshly damaged tissue . Coral five (from *Porites lutea*) was not sampled on 06/02/2017 at 12:00AM. Coral fragments were placed into RNAlater in the field then immediately placed on dry ice. Four water samples were also taken, two on 3 June at 1200h and 3 Jun at 2400h. These were obtained by filtering 120 mL of seawater through a 0.22 micron filter (Millipore). The filter was then placed into RNAlater and frozen on dry ice. All samples were stored at -80 °C until processing.

RNA/DNA extraction and RNAse/DNAse treatment

DNA and RNA were extracted simultaneously from all 89 coral samples using the Qiagen RNeasy PowerMicrobiome Kit. A fragment of approximately .25 g of coral mucus/tissue/skeleton from the original coral sample was used for the extraction, which was performed according to the manufacturer's instructions. This resulted in an aliquot of both RNA and DNA, which was divided into two separate aliquots, one designated for DNA and one for RNA. Two separate Qiagen RNeasy PowerMicrobiome Kits were used for extractions and one blank extraction (no coral fragment) was performed on each kit, for a total of two extraction blanks. The four seawater samples were extracted using the Qiagen DNeasy PowerSoil Kit, resulting in an aliquot of DNA only. The water filters were placed directly in the PowerBead tube and the extraction performed according to the manufacturer's instructions. After extraction, all DNA and RNA extracts were stored at -80°C until further processing.

DNA was purified using 1 µl of RNase I_f (New England BioLabs Inc.) added to 20 µl of template DNA, which was then incubated at 37 °C for 20 minutes to digest RNA, followed by 15

minutes at 70 °C to inactivate the enzyme. DNA samples were frozen at -20 °C following RNase I_f treatment. To prepare the RNA for cDNA synthesis, 2.0 µl DNase master mix was added to 1.4 µl of RNA template diluted in 12.6 µl of nuclease-free PCR water and incubated according to the manufacturer's protocol. Reverse transcription was performed immediately following DNase treatment using the iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.). Reactions were performed according to the manufacturer's protocol. Samples were incubated at 25 °C for 10 minutes, 46 °C for 50 minutes, then 95 °C for 1 minute to generate the cDNA libraries. All samples were stored at -20 °C following treatment.

16S rRNA amplicon analysis (DNA and cDNA)

PCR was performed on all purified coral and seawater DNA (representing community composition) and coral cDNA (representing relative transcriptional activity of community members) samples. The V4 region of the 16S rRNA gene was amplified using primers F515 and R806[40] equipped with barcodes and Illumina-specific adapters[41]. Reactions included 12.5 µl GoTaq Hot Start Green Master Mix (Promega), 10.5 µl Nuclease free PCR water, 0.5 µl BSA (New England BioLabs Inc.), 0.25 µl each of the Forward and Reverse primer, and 1 µl of DNA/cDNA template. Unsuccessful amplifications were attempted again with modified ratios; nuclease free water was reduced to 9.5 µl and the DNA template was increased to 2 µl. For the coral and seawater DNA samples, PCR conditions were as follows: 30 cycles of 94 °C for one minute, 55 °C for two minutes, then 68 °C for 90 seconds followed by an addition annealing period of ten minutes at 68 °C. For the cDNA samples, PCR conditions added an initial denaturing step for two minutes at 94 °C, then continued as for the DNA for 25 cycles instead of 30. PCR amplicons were verified using gel electrophoresis and quantified using Qubit (Life Technologies) and pooled at equimolar concentrations. The amplicon libraries were then cleaned using Diffinity RapidTip PCR purification tips (Diffinity Genomics, NY). Finally, amplicons were sequenced across six different runs using a paired-end Illumina MiSeq 500 cycle kit with 5% PhiX to increase read diversity. The first three runs included DNA samples and the second three contained the cDNA samples, with time points and coral heads distributed randomly across the runs.

Raw fastq files were imported into QIIME2[42] and demultiplexed using the q2-demux plugin. Reads were run through the DADA2[43] (using q2-dada2) pipeline to identify Sequence

Variants (SVs) trimming 70 base pairs off the 5' end of the sequences and then truncating to 150 base pairs. The q2-feature-classifier was used with the SILVA pre-trained classifier (silva-132-99-515-806-nb-classifier) to assign taxonomy to the SVs. Following taxonomic assignment all sequences assigned as chloroplast, mitochondria, or not within the Bacteria and Archaea domains were removed from further analysis. Alpha diversity was measured via the Chao1 richness estimator and the Shannon diversity index using the q2-diversity plugin. Plots of alpha diversity were produced using the Phyloseq[44] package in R (version 3.6.2) at the microbial SV level. Beta diversity was assessed using square-root transformed Bray-Curtis dissimilarity matrices calculated in PRIMER7 (Primer-E Ltd.). Non-metric Multidimensional Scaling (NMDS) plots were generated to visualize inter-sample variation in PRIMER7. Bar plots showing relative abundance/transcriptional activity were produced in Microsoft Excel at the microbial species level or higher by pooling all samples by dataset (DNA or cDNA), timepoint, and species.

Statistical analysis

To investigate differences between time points, alpha and beta diversity were calculated for DNA and cDNA datasets independently, rarefying to 7,598 and 3,038 sequences, respectively. Analyses were done individually for each coral species. Due to no significant differences existing between the 2400h and the 1200h samples on Days 1 and 2,for beta diversity analyses, the six time points from which samples were taken were grouped based on the time of day at which the sample was taken in order to increase statistical power. The Day 1 – 2400h and Day 2 – 2400h samples were pooled together and the Day 1 – 1200h and Day 2 – 1200h samples were pooled together to create four time points: 2400h, 0600h, 1200h, and 1800h (called pooled). 0600h and 1800h were each sampled on only one day and therefore, are their own time point. For alpha diversity, significance was calculated based on the six sample times separately. 2400h and 1200h time points on different days were treated separately, resulting in six time point groups (called unpooled). Pairwise comparisons were performed when the global test was significant. Significant differences in alpha diversity at the microbial SV level between unpooled time points were analyzed using a Kruskal-Wallis test in QIIME2[42] using the q2-diversity plugin to determine if certain time points contained more or less diverse communities within the DNA or cDNA dataset. Significant differences in beta diversity (community composition and dispersion) between pooled time points were determined using a Permutational Multivariate

Analysis of Variance (PERMANOVA) and Homogeneity of Multivariate Dispersions (PERMDISP) tests, respectively. These tests were done at both the microbial SV and microbial species level. To further look for differences between pooled time points, a Canonical Analysis of Principal Coordinates (CAP) was performed at the microbial species level with the axes constrained by time. The m-value was chosen to be greater than or equal to two. The CAP also was used to determine if the different groups could be accurately predicted using leave-one-out cross validation[45]. Pillai's trace and Roy's greatest root statistics were calculated for the CAP. Additionally, a PERMANOVA was used to determine if differences exist between the coral species (DNA and cDNA separately) at the microbial SV level. A PERMANOVA was also used to determine if the seawater and coral DNA were significantly different. All beta diversity analyses were performed in PRIMER7.

When directly comparing DNA and cDNA, a rarefied sequence depth of 3,038 was used for both datasets (DNA/cDNA dataset). Analyses were done individually for each coral species with all data within a species pooled, independent of the different time points. To determine if there were significant differences in either alpha diversity metric (Shannon or Chao1) between the DNA and cDNA datasets, a two-tailed t-test was performed in R. Both a PERMANOVA (composition) and PERMDISP (dispersion) test were used to analyze differences in beta diversity between DNA and cDNA communities. Then, significant differences in beta diversity between DNA and cDNA at the pooled time points were tested. Differences between DNA and cDNA were examined by groups based on both coral species and pooled time point using a PERMANOVA and PERMDISP to determine if temporal patterns were similar for DNA and cDNA through time.

Using the same rarefaction depth (3,038) for both DNA and cDNA datasets, SVs were identified as unique if they were only present in the DNA (not cDNA) or only present in cDNA (not DNA). Additionally, to detect transient SVs within each dataset (DNA or cDNA) and within each coral head (1-5 for *Porites lutea*, 6-10 for *Porites cylindrica*, and 11-15 for *Pocillopora damicornis*), the number of SVs present in a certain number of unpooled time points (i.e. how many were in one out of six time points, two out of six time points, etc.) was calculated. The percentage of total SVs per coral head that was in a certain number of unpooled time points was calculated. From this, the SVs that were present in all unpooled time points of a single coral head were identified. Seawater samples were not included in this analysis.

RESULTS

Alpha diversity

Details about the number of samples retained in each dataset after rarefaction and the number of SVs in each dataset are contained in Appendix B. There were no significant differences in either alpha diversity metric for the DNA or cDNA datasets through time (unpooled). Unpooled time points represent the six individual times that samples were taken. Neither alpha diversity metric had consistent patterns in alpha diversity through time across coral species (Figure 1, Figure 2).

Alpha diversity differed significantly between the DNA dataset and cDNA dataset for *Porites cylindrica* (Chao1 $p = 0.019$, Shannon $p < 0.0001$) and *Pocillopora damicornis* (Chao1 $p = 0.018$, Shannon $p = 0.0005$) for all unpooled time points. For both corals, Shannon diversity and Chao1 richness were lower in the cDNA dataset compared to the DNA dataset (Appendix C). *Porites lutea* alpha diversity did not differ significantly between DNA and cDNA datasets (Chao1 $p = 0.47$, Shannon $p = 0.57$).

Table 1. Differences in beta diversity based upon square-root transformed Bray Curtis dissimilarities. 2400h (Day 1 and Day 2) and 1200h (Day 1 and Day 2) were combined for this analysis and 0600h and 1800h only have one day of samples. Significant differences in time points (*p-value < .05) were detected in the DNA dataset for *Pocillopora damicornis* at the microbial species level (see Appendix D), but not in the cDNA dataset. No significant differences were detected at the microbial sequence variant level in either the DNA or cDNA datasets, or between time points at microbial species level for cDNA samples (data not shown).

	Porites lutea	*Porites cylindrica*	*Pocillopora damicornis*
PERMDISP	0.53	0.81	**0.03***
PERMANOVA	0.41	0.73	**0.04***

Beta diversity

All beta diversity analyses were performed separately for each coral species and with time points with the 2400h and 1200h from the separate days pooled. PERMANOVA and PERMDISP analysis indicated global significance in beta diversity (community composition or dispersion) across all pooled time points at the microbial species level in the *Pocillopora damicornis* DNA dataset (PERMANOVA $p = 0.04$, PERMDISP $p = 0.03$), but not in the *Porites*

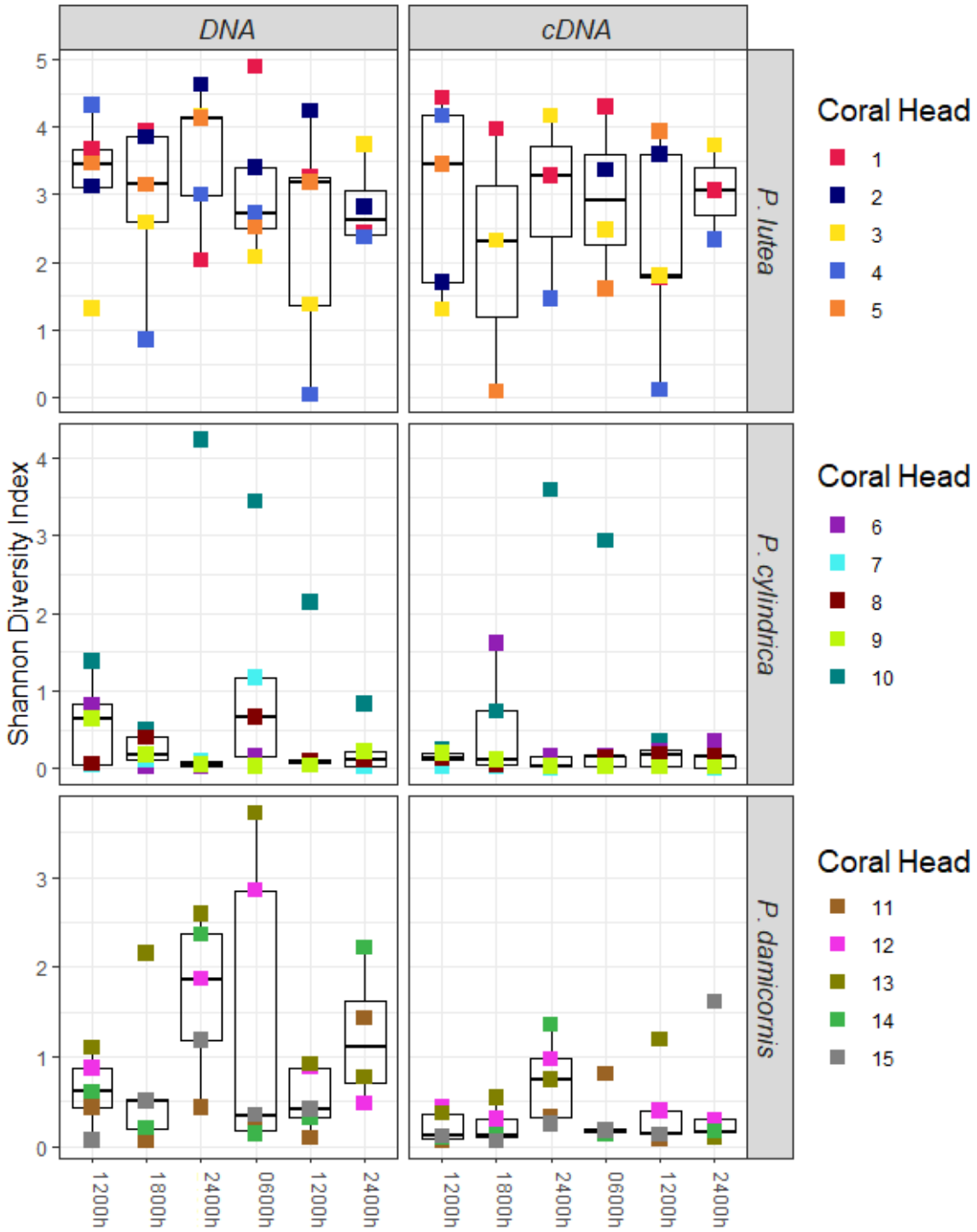

Figure 1. Sampling time does not have a patterned effect on Shannon alpha diversity. The plot shows the Shannon diversity index (alpha diversity) for three coral species, *Porites lutea* (coral heads 1-5), *Porites cylindrica* (coral heads 6-10), and *Pocillopora damicornis* (coral heads 11-15) at six different timepoints across 48 hours (times left to right are in sequential order, repeated numbers are on separate days). No significant differences were detected between time points, with (shown above) or without 2400h and 1200h pooled (data not shown).

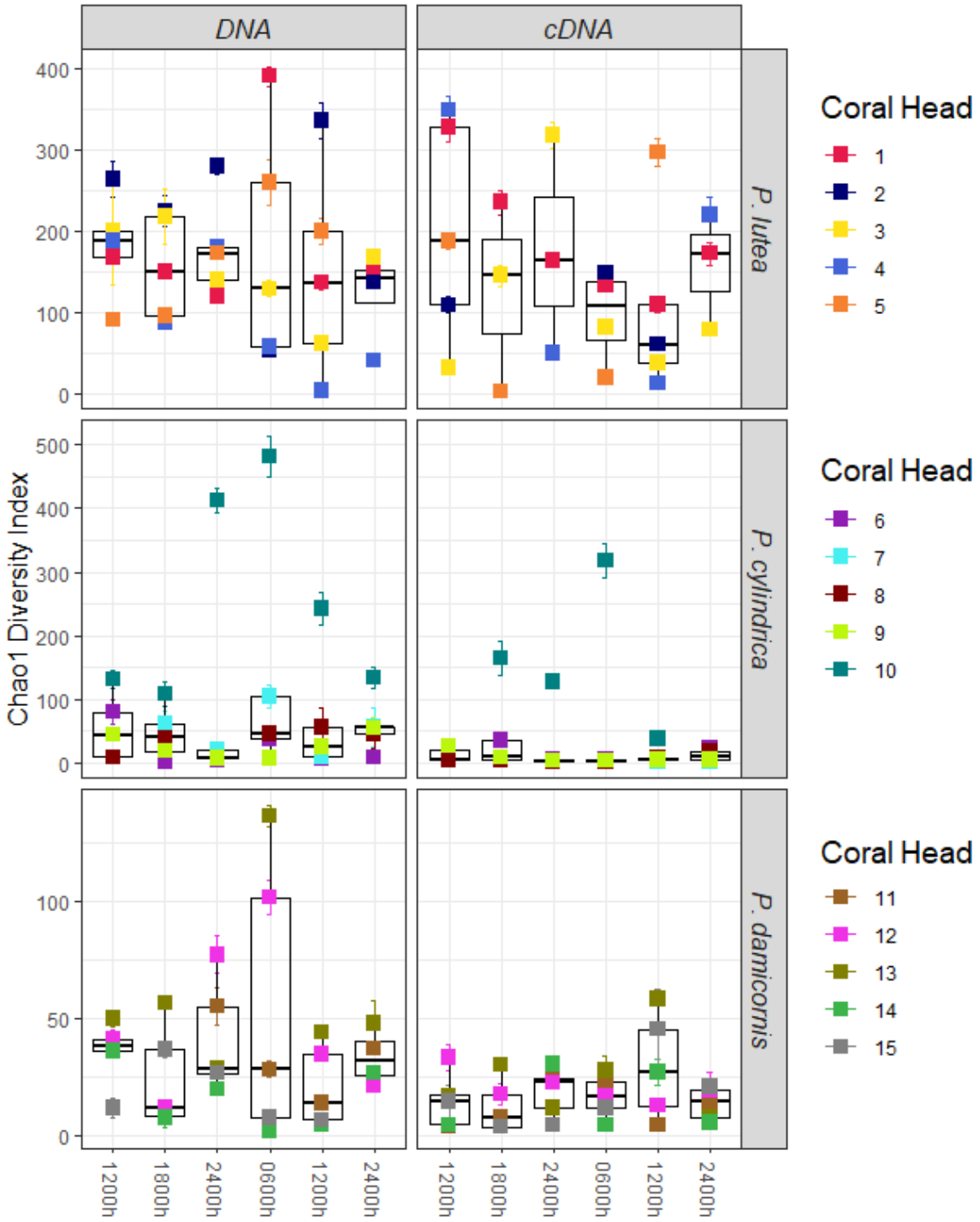

Figure 2. The plot Chao1 diversity index (alpha diversity) for three coral species, *Porites lutea* (coral heads 1-5), *Porites cylindrica* (coral heads 6-10), and *Pocillopora damicornis* (coral heads 11-15) at six different timepoints across 48 hours (times left to right are in sequential order, repeated numbers are on separate days). No significant differences were detected between time points, with or without 2400h and 1200h pooled (pooled data not shown).

lutea (PERMANOVA p = 0.41, PERMDISP p = 0.53) or *Porites cylindrica* (PERMANOVA p = 0.73, PERMDISP p = 0.82) DNA datasets (Table 1). Since the global test was significant for the *Pocillopora damicornis* DNA dataset, pairwise tests were performed between unpooled time points and revealed that significant differences in community composition existed between 2400h and 1200h (p = 0.007), and significant differences in dispersion existed between 2400h and 1200h (p = 0.024) and 0600h and 1200h (p = 0.001) (Appendix D). Patterns between time points are not obvious, even in *Pocillopora damicornis* based on an nMDS (Figure 3),

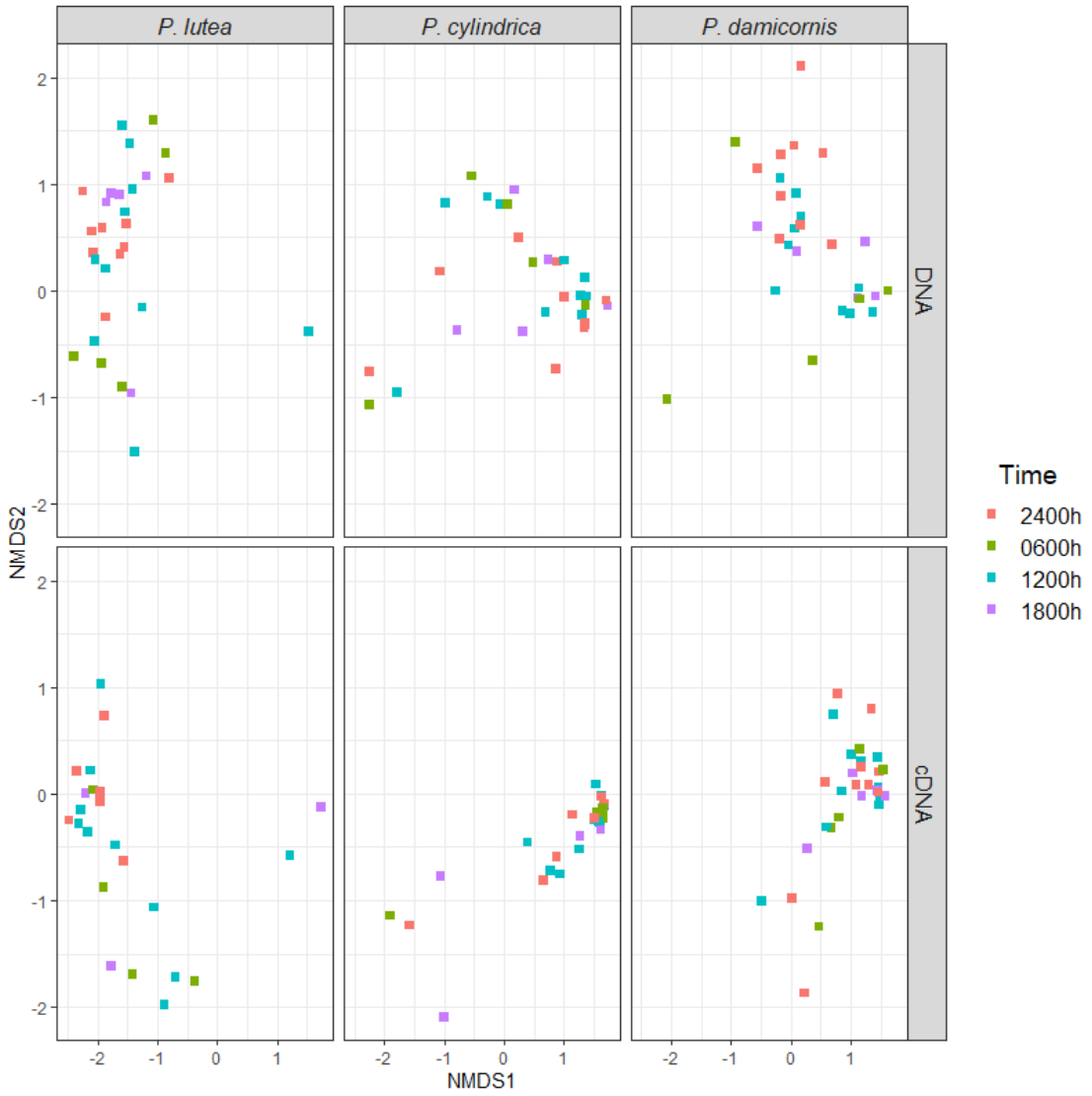

Figure 3. Time point variation between time points is not seen for any coral species between either DNA or cDNA datasets based on non-metric multidimensional scaling analysis of square-root transformed Bray Curtis dissimilarities at the microbial species or higher level.

so to further test for significant variation between time points, CAP analyses were performed. The *Pocillopora damicornis* CAP analysis of the DNA dataset was the only significant CAP analysis ($p = 0.026$), indicating that this dataset explained a significant amount of the overall variation between samples. The CAP plot for the DNA dataset of *Pocillopora damicornis* showed that 2400h and 0600h samples cluster separately and a cluster with 1200h and 1800h is seen (Appendix E.1). Leave-one-out cross validation was only performed for this significant CAP analysis and had 46.1% accuracy, with 2400h performing the best (Appendix E.2). When evaluating differences in beta diversity across all pooled time points at the microbial SV level; we did not detect significant variation using PERMANOVA, PERMDISP, or CAP for any of the coral species, despite *Pocillopora damicornis* being significant at the microbial species level.

Surprisingly, cDNA-based microbiome composition and dispersion did not differ significantly between time points for any of the three coral species and this is seen on the nMDS (Figure 3). Within *Pocillopora damicornis*, which exhibited significant variation in microbial species composition across all time points (DNA), neither composition ($p = 0.89$) nor dispersion ($p = 0.64$) varied significantly based on cDNA sequences. The CAP analysis also did not represent a significant amount of variation (Trace statistic: $p = 0.49$; Roy's greatest root, $p = 0.11$). Neither *Porites lutea* nor *Porites cylindrica* composition or dispersion varied significantly at either the microbial SV or species levels.

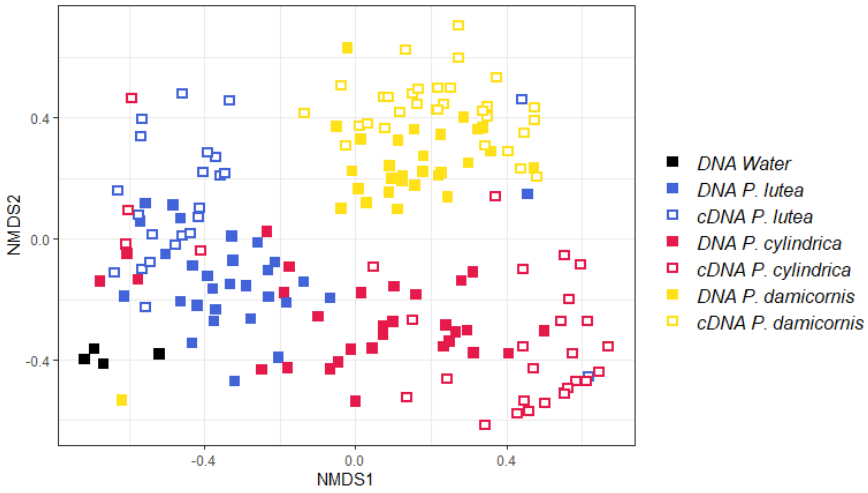

Figure 4. 16S rRNA gene (DNA) and transcripts (cDNA) cluster separately within each coral species. The plot compares the taxonomic composition of DNA and cDNA datasets based on non-metric multidimensional scaling analysis of square-root transformed Bray Curtis dissimilarities at the microbial sequence variant level.

Seawater microbiomes clustered separately from coral microbiomes, and coral microbiomes clustered according to both the type of data (DNA or cDNA) and coral species (Figure 4). A PERMANOVA indicated significant differences in community composition between seawater and coral DNA datasets (p = 0.0001) and between DNA and cDNA datasets when data from all coral species were pooled (p = 0.0001). Significant differences also existed

between the coral species (pairwise comparison) based on both the DNA (p = 0.0001) and cDNA datasets (p = 0.0001). A PERMDISP indicated that inter-sample dispersion differed significantly between *Porites lutea* and the other two corals (vs. *Porites cylindrica* p = 0.0001, vs. *Pocillopora damicornis* p = 0.0001), but not between *Porites cylindrica* and *Pocillopora damicornis* (Table 2). This was true for both DNA (p = 0.29) and cDNA (p = 0.89) datasets (Table 2).

Table 2. Pairwise differences in beta diversity between coral species based upon square root transformed Bray Curtis dissimilarities. Dispersion was not significantly different between *Porites cylindrica* and *Pocillopora damicornis*.

PERMANOVA		**DNA P-value**	**cDNA p-value**
Porites lutea	*Porites cylindrica*	**0.0001***	**0.0001***
Porites lutea	*Pocillopora damicornis*	**0.0001***	**0.0001***
Porites cylindrica	*Pocillopora damicornis*	**0.0001***	**0.0004***
PERMDISP			
Porites lutea	*Porites cylindrica*	**0.0001***	**0.0001***
Porites lutea	*Pocillopora damicornis*	**0.0001***	**0.0001***
Porites cylindrica	*Pocillopora damicornis*	0.30	0.89

In general, DNA and cDNA clustered more distinctly at 2400h and 1200h but were not separated on a nMDS at 0600h and 1800h (Appendix F). When testing for differences between DNA and cDNA within each coral species individually with all six time points within a species pooled, PERMANOVA indicated significant differences in community composition between the DNA and cDNA datasets at the microbial SV (*Porites lutea* p 0.0001, *Porites cylindrica* p = 0.0016, *Pocillopora damicornis* p = 0.0001) and microbial species level (*Porites lutea* p 0.0001, *Porites cylindrica* p = 0.0012, *Pocillopora damicornis* p = 0.0001) for all three coral species. However, dispersion between DNA and cDNA datasets only differed significantly for *Pocillopora damicornis* at both the microbial SV (p = 0.012) and microbial species level (p = 0.008) (Appendix G).

Unique SVs

The three coral species shared 146 DNA SVs out of 3,807 and 70 cDNA SVs out of 2,440 in the DNA/cDNA dataset, or about 3.8% and 2.9%, respectively. There were 2,753 SVs unique to DNA and 1,386 unique to cDNA, with 1,054 SVs shared between the two datasets. When unique SVS were examined between each time point within each individual coral head, a large percentage of SVs, in both DNA and cDNA datasets, were detected at only one of the six timepoints (>66-70%; Tables 3 and 4).

Table 3. Percentage of 16S rRNA gene (DNA) sequence variants detected in 1,2,3,4,5 or all 6 datasets (timepoints) for each coral head (1-15). Coral heads for which datasets are available from only 5 (*) timepoints are marked. Average abundances of SVs present in all six time points are displayed in Appendix H.1.

	Coral														
Number of	*Porites lutea*					*Porites cylindrica*					*Pocillopora damicornis*				
timepoints	1	2	3	4	5*	6	7	8	9	10	11	12	13	14	15*
1/6	86.1	85.9	75.5	86.0	83.6	64.1	80.0	64.2	53.3	70.3	72.1	67.2	88.5	70.1	80.5
2/6	7.2	7.8	14.0	9.4	9.7	18.5	10.3	23.9	23.3	16.9	14.0	30.5	4.8	19.5	5.2
3/6	2.9	3.2	4.9	2.1	3.4	8.7	5.5	6.4	10.0	8.8	6.6	6.3	2.8	2.6	5.2
4/6	1.7	1.2	3.0	1.3	2.5	7.6	2.8	3.7	8.9	3.0	1.5	3.9	1.4	3.9	3.9
5/6	0.9	1.2	2.0	1.0	0.8	0.0	0.0	0.9	2.2	0.8	2.2	1.6	2.5	1.3	5.2
6/6	1.3	0.7	0.7	0.2	N/A	1.1	1.4	0.9	2.2	0.3	3.7	7.0	0.0	2.6	N/A

Table 4. Percentage of 16S rRNA transcript (RNA) sequence variants detected in 1,2,3,4,5 or all 6 datasets (timepoints) for each coral head (1-15). Coral heads for which datasets are available from only 4 (*) or 3 (**) timepoints are marked. Average abundances of SVs present in all six time points are displayed in Appendix H.2.

	Coral														
Number of	*Porites lutea*					*Porites cylindrica*					*Pocillopora damicornis*				
timepoints	1	2**	3	4*	5*	6	7	8	9	10	11	12	13	14	15
1/6	76.5	84.1	81.9	83.7	87.1	90.7	68.6	76.0	81.0	73.5	75.4	66.3	73.5	78.3	85.1
2/6	12.3	17.9	11.6	12.9	11.7	5.3	12.5	8.0	7.1	18.2	15.9	9.6	14.7	10.1	4.5
3/6	4.7	6.0	4.1	3.2	1.0	1.3	12.5	4.0	4.8	6.8	0	7.2	4.4	1.5	0
4/6	2.9	N/A	2.1	0.2	0.2	0	0	4.0	2.4	0.8	1.5	1.2	0.7	4.4	6.0
5/6	2.1	N/A	0	N/A	N/A	1.3	0	0	2.4	0.6	2.9	3.6	2.9	0	0
6/6	1.6	N/A	0.3	N/A	N/A	1.3	6.3	8.0	2.4	0.2	4.4	12.1	3.7	5.8	4.5

A very low percentage of SVs were present across all sampling points within a specific coral head. This number ranged from 0% to 3.7% within the DNA dataset (Table 3) and from 0.2% to 12.1% within the cDNA dataset (Table 4). Six out of 28 SVS in the DNA dataset and

eight out of 33 SVs in the cDNA dataset that were present across all time points in a single coral head were classified as bacteria of the genus *Endozoicomonas* (Gammaproteobacteria). *Endozoicomonas* also represented the SV with the largest relative abundance in 11 of 15 coral heads in the DNA (Appendix H.1) and 12 of 15 coral heads in the cDNA (Appendix H.2) datasets. Most of these SVs identified were high abundance (>1%), but there were also several low abundance SVs present in every time point within some of the individual coral heads (Appendix H). *Porites lutea* was the only coral with low abundance SVs (besides *Endozoicomonas)* that were consistently detected in all time points across the five coral heads, but only within the DNA. *Vibrio* spp. (assigned *cholerae*), *Aeromonas,* and *Novosphingobium* were present in at least three of the five *Porites lutea* coral heads at relative abundances of 2-5% of the community. Within *Pocillopora damicornis, Phycisphaera* (Planctomycetes) was present in two coral heads within the DNA dataset and one coral head within the cDNA dataset at abundances of less than 1%.

Community composition and transcriptionally active members

Porites cylindrica and *Pocillopora damicornis* samples were dominated by *Endozoicomonas,* whose abundance ranged from 60-95% of the DNA and cDNA datasets (Figure 5). *Porites lutea* microbiomes contained a smaller but still substantial percentage of *Endozoicomonas but* were distinguished by high abundances (2-25%) of a second gammaproteobacterial genus *Oleiphilus* (Figure 5). *Porites lutea* also contained a larger percentage (up to 50% of total sequences) of rare microbal species compared to the other two coral species, with "rare" defined as a taxon (grouped at species level or higher) accounting for <0.06% of total sequences (Figure 5). Seawater microbiomes contained dominant taxa differing from those in the corals, namely two members of the Order Flavobacteriales, and also contained a large percentage (~ 50%) of rare taxa (species level or higher).

Six taxa differed in abundance between DNA and cDNA across all time points (Figure 5). *Vibrio cholerae, Pseudomonas, Aeromonas,* unclassified Gammaproteobacteria, *Novophingobium,* and rare taxa had a higher relative abundance within DNA than cDNA for all three coral species. *Vibrio cholerae, Aeromonas,* and *Novophingobium* taxa were almost completely absent from the cDNA dataset. However, caution should be taken when looking at the presence of *Vibrio cholerae,* as *Vibrio* spp. are hard to classify using short fragments of

the16S rRNA gene. Midichloriaceae MD3-55 was only present in abundances greater than five copies in *Porites lutea*, but represented the only taxon with a higher relative abundance in cDNA than in DNA. While community DNA composition differed significantly from the composition of the transcript pool, we did not detect clear cyclical shifts in the most abundant microbial members in either DNA or cDNA composition that correspond to transitions between conditions over the diel cycle.

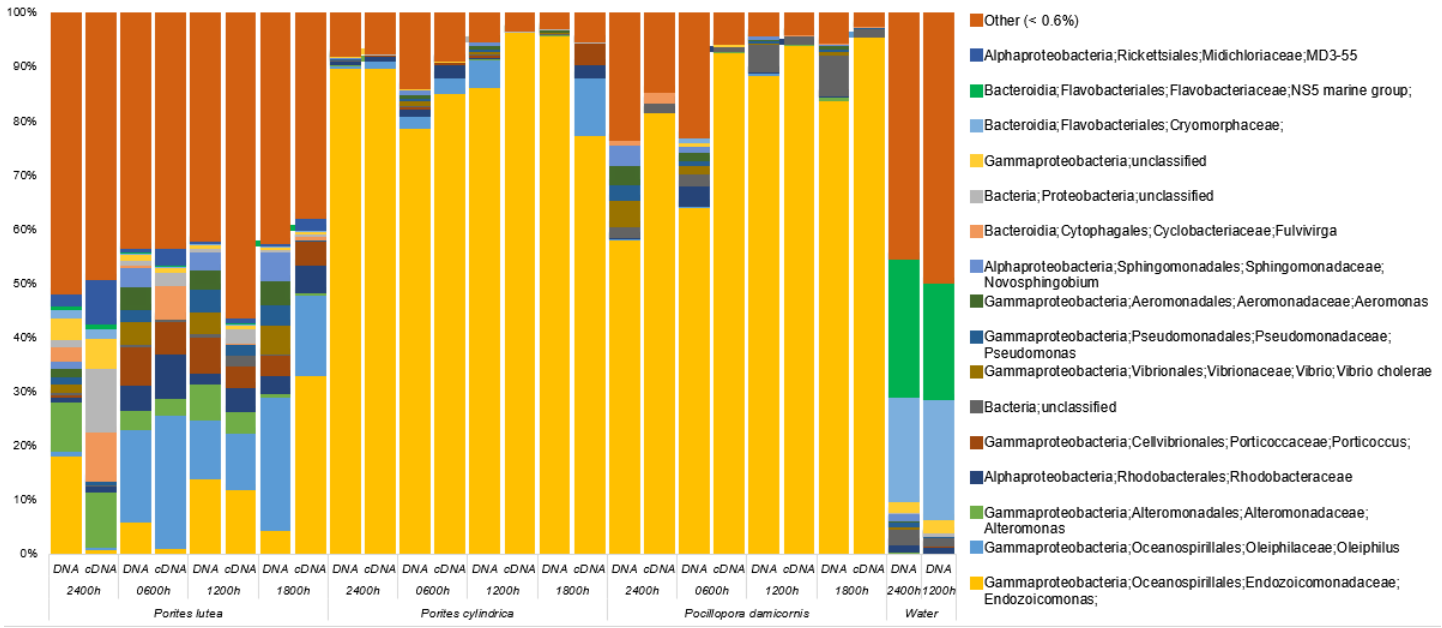

Figure 5. Small compositional differences exist between the 16S rRNA gene (DNA) and transcript (cDNA) datasets. The plot shows mean percentage abundance of microbial taxa (grouped at species level or higher) in DNA and cDNA datasets. All 2400h (Day 1 and Day 2) and 1200h (Day 1 and Day 2) time points were pooled for this analysis and there was only one day of samples for 0600h and 1800h.

DISCUSSION

To better understand coral microbiome dynamics, it is important to consider short-term variation in composition and relative transcriptional activity of community members. Data from 89 total coral heads (15 coral heads sampled at six time points with the exception of coral head 5 on 3 June at 2400h) from three different coral species showed that daily dynamics of the coral microbiome are genus-specific with regards to community composition, but the transcript pool remains stable across species. There were no significant differences between time points in the transcript pool for any of the three coral species, indicating that the microbiome members that, are transcriptionally active, at least in *Pocillopora damicornis,* are changing less over time than overall community composition. There were no significant differences between time points across coral species or dataset (DNA or cDNA). Higher alpha diversity in the DNA dataset compared to the cDNA dataset suggests that transient bacteria that are not transcriptionally active may be responsible for changes in the overall composition in *Pocillopora damicornis*. *Pocillopora damicornis* community composition changed significantly, showing that its composition is more variable through time than the composition in *Porites lutea* and *Porites cylindrica,* which did not exhibit patterned changes. This shows that in spite of living in a very dynamic environment, *Porites* corals do not show consistent microbiome changes across diel time periods.

The lack of significant short-term variation in *Porites lutea* and *Porites cylindrica* microbiomes does not indicate that the microbial communities are not dynamic. The lack of significance could indicate that there were no patterned or consistent changes between time points or that the higher abundance microbes stay stable and override the signal of the changing lower abundance microbes. In fact, all three corals contained a large percentage of lower abundance, transient microbes that were only present in a single time point (i.e. less than 6 hours). This indicates that a very large portion of the coral microbiome is made up of dynamic, transient bacteria, but that there is no consistent diel pattern to these changes that we could detect.

These dynamic bacteria, which represent a majority of the coral microbiome, have been called the "environmentally responsive community"[46]; however, this study found no diel patterns

in transient members that might correspond to changes in environmental conditions occurring day to night[14] . This indicates that the transient nature of these microbes may not be linked to diel variation in abiotic factors. Additionally, there was little overlap in these transient microbes between coral heads from the same coral species, showing that these microbes may be truly random members of the community.

These results also have implications for understanding the coral core microbiome, defined as microbial members that are stable and consistent across a system[47]. There were 146 SVs from the DNA dataset that were shared across the three coral species, a number similar to the one found in a study investigating the coral core microbiome[48]. Additionally, 70 SVs were transcriptionally active across all three coral species. However, while present across all species, when individual coral heads within a single species and time point are pooled, most of these shared SVs were not present in the coral community at all time points. This provides additional evidence that these microbiomes are dynamic. In fact, *Endozoicomonas* is the only microbial taxon that was consistently present in both datasets across all time points in all coral heads (except coral head 13 DNA). When looking within individual coral species, only *Porites lutea,* which had the lowest relative abundance of *Endozoicomonas,* had several other microbial species that were consistently present across all time points in most (at least 3 out of 5) of its coral heads. Within DNA dataset, this included *Vibrio* spp. (assigned *cholerae*), *Aeromonas,* and *Novosphingobium*. Within the cDNA dataset for *Porites lutea,* no microbial taxa were present in more than 2 of the 5 coral heads across all time points. Overall, this shows that the core members of the microbiome may not be stable through time and that some species may have higher diversity in their species-specific core members.

Acclimation to changing temperatures has been shown to play a large role in microbiome response to stress[19,25]. Although studies of phenotypic plasticity often focus on multicellular organisms, bacterial cells are also capable of displaying diverse phenotypes[49]. It is possible that different coral hosts select for more plastic bacteria, and that this allows for greater stability through changing conditions. This study found a large percentage of the microbial community consisting of low abundance transient microbes, but that the highly abundant microbes largely stay consistent in proportional abundance throughout the diel cycle. Complete dominance by a single microbial species may indicate an inflexibility suggesting an inability to adapt to change, for example as seen in a study of *Pocillopora verrucosa* that showed that *Endozoicomonas*

dominated the coral community both before and after coral bleaching[50]. Further investigations are needed to determine the effect of microbiome communities on host acclimation ability.

Several studies have shown that microbiomes can acclimate to temperatures, over both long and short timescales. Long term, the microbiome of corals that experience large seasonal temperature ranges shifts more quickly with heat stress during the winter (12 hours) than during the summer (48 hours), indicating that the microbiome acclimatizes to its current conditions[51]. Over a shorter time scale of hours, coral microbiomes that have been exposed to environmental variation for 17 months remain more stable when exposed to heat stress of hours than did the microbiomes of corals that have not been exposed to variation[35]. The corals in our study live in the same small patch of a shallow reef, which likely exhibits high environmental variation[14]. Since all corals likely experience the same conditions and microbiome stability has been shown in corals acclimated to variable environments[35], the microbiome stability of the two *Porites* corals could indicate that *Porites* corals have a larger capacity to protect and regulate their beneficial microbiomes under shifting conditions than does *Pocillopora damicornis,* which could contribute to the increased resistance of *Porites spp*. to heat stress[39].

The ability to resist and/or acclimate to stress is likely host-specific and the differing responses seen in the microbiomes of these three coral species over short time scales could be indicative of this. Microbiome changes under bleaching and high temperature conditions differ largely based on what coral species is being examined[52], indicating that host taxonomy plays a large role in the microbial response to stress. The extent of the coral microbiome's ability to remain stable over the highly variable conditions of a diel cycle is likely dictated by host as well. *Porites* corals are relatively resistant to bleaching and their microbiome composition remains stable over a diel cycle in this study. In contrast, *Pocillopora* bleaches more quickly but is one of the first corals to recover[39] and is shown here to experience microbial composition shifts. Differences in host genus are likely a driver of these differences. It has been shown that presence of different strains of *Symbiodinium* can influence the stability of the microbiome during heat stress in the same coral species[53]; however, *Symbiodinium* strains have also been shown to have host specificity[54]. More work is needed to determine the distinct roles of host species, *Symbiodinium* strain, and microbiome stability in coral stress resistance.

As a cautionary note, our study did not take measures of environmental conditions at the sampling times, so the compositional changes seen in *Pocillopora damicornis* cannot be directly

correlated to chemical or physical parameters, but these parameters are predicted to vary considerably during diel cycles due to differences in photosynthesis and light levels. Future studies investigating diel microbiome shifts would profit from collecting environmental measurement (including of temperature, pH, dissolved and particulate organic carbon and nitrogen) in addition to the microbial samples. This way, microbiome shifts, or the lack there of can be directly coupled with changes in the environment.

The data collected in this study show how dynamic coral microbiomes can be. Several SVs were consistently present across coral heads and time points in the cDNA dataset, indicating transcriptional activity of those microbes, but not their consistent presence across the DNA dataset. There were also additional SVs unique to the cDNA dataset that were transient members of the transcript pool. It is likely that the microbes are low abundance members of the microbiome, but are active members, so they are detected in the cDNA dataset, but not the DNA dataset. This lack of detection could be due to the random sampling of sequences during rarefaction, such that rare members are randomly included or excluded. Discrepancies between DNA and cDNA datasets for rare, but potentially important, members of the microbiome could also be due to PCR and sequencing biases driven by variation in template concentration[55].

Our data also show that coral microbiomes appear more similar between corals that share morphology, rather than taxonomic relatedness. Although we only have one mounding coral and two branching corals and cannot adequately assess the role of taxonomy or morphology in structuring the coral microbiome, it is interesting that the microbiome of *Porites cylindrica* (a branching coral) more closely resembles that of *Pocillopora damicornis,* another branching coral instead of *Porites lutea* (a more closely related species, but a mounding coral). Notably, both corals had a higher *Endozoicomonas* abundance compared to *Porites lutea.* Different "sites" (mucus, tissue, skeleton) in the coral body offer different habitats and have distinct microbiomes[56] – it is therefore possible that microhabitat variation is greater between corals of differing overall morphologies, compared to corals with similar structures.

CONCLUSION

Overall, this study found that coral microbiomes dynamics are species-specific over a diel cycle, with *Pocillopora damicornis* microbiomes experiencing compositional and dispersion differences between time points while the microbiomes of both *Porites* corals remained stable for these parameters. These species-specific differences could contribute to the increased resilience to bleaching of *Porites* corals and suggest that the *Porites* microbiome may have a larger capacity for acclimating to environmental conditions compared to *Pocillopora,* but since this study did not measure changes environmental factors at sampling time, microbiome shifts cannot be directly coupled to environmental variation. Despite compositional change, the transcript pool does not differ significantly over time points for all three coral species, indicating that transient but not transcriptionally active bacteria may be responsible for the overall compositional shifts in *Pocillopora damicornis*. Despite an overall lack of significance between time points, the coral microbiome is very dynamic, mainly with regard to lower abundance community members. Many microbiome members were detectable at only one time point, with no more than 15 SVs being present in all time points in a particular coral head. In fact, *Endozoicomonas* was the only microbial taxa present across all time points in many of the coral heads (8 for the DNA dataset and 7 for the cDNA dataset). This variability has implications for defining a coral's core microbiome, indicating a need to consider short term (hour to hour) microbiome changes to truly understand dynamics of the coral microbiome and how it may affect a coral's ability to resist stressors.

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APPENDIX A

Sampling Scheme. Samples were taken from three coral species across six time points within a 48-hour period. Five coral heads were sampled for each coral species, with the exception of *P. lutea*.

Species	***Porites lutea***					***Porites cylindrica***					***Pocillopora damicornis***					**Water**
Coral Head	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Day 1 – 1200h	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Day 1 – 0600h	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Day 1 – 2400h	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Day 2 – 0600h	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Day 2 – 1200h	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	2
Day 2 – 2400h	X	X	X	X		X	X	X	X	X	X	X	X	X	X	2
Total	**6**	**6**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**4**

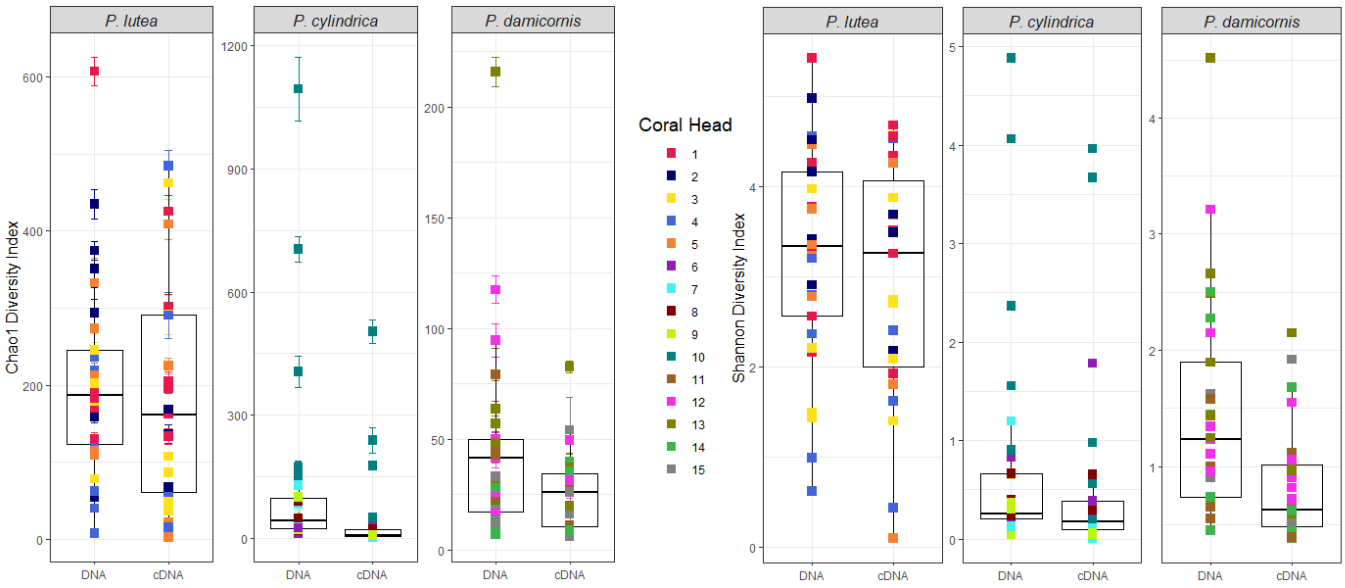
APPENDIX B

Samples and sequence variants (SVs) remaining in each data set after quality control and rarefaction. After sequencing and rarefying, 93% of samples were retained in both DNA and cDNA data sets and 96% were retained in the combined data set.

Dataset	Rarefaction Depth	Samples Retained	SVs Present
DNA	**7598**	**83/89**	**4958**
Porites lutea		27/29	3424
Porites cylindrica		30/30	1940
Pocillopora damicornis		26/30	701
cDNA	**3038**	**83/89**	**2369**
Porites lutea		23/29	1941
Porites cylindrica		30/30	588
Pocillopora damicornis		30/30	296
DNA/cDNA	**3038**	**171/178**	**5193**
Porites lutea		52/60	4017
Porites cylindrica		60/60	1560
Porites damicornis		59/60	806

APPENDIX C

Microbiome sequence diversity differs between the DNA and cDNA datasets. The figure shows Chao1 (left) and Shannon (right) alpha diversity for the 16S rRNA gene (DNA) and transcripts (cDNA) datasets from the three corals species. Differences are significant for *Porites cylindrica* (p = 0.019) and *Pocillopora damicornis* (p = 0.0005) but not *Porites lutea*.



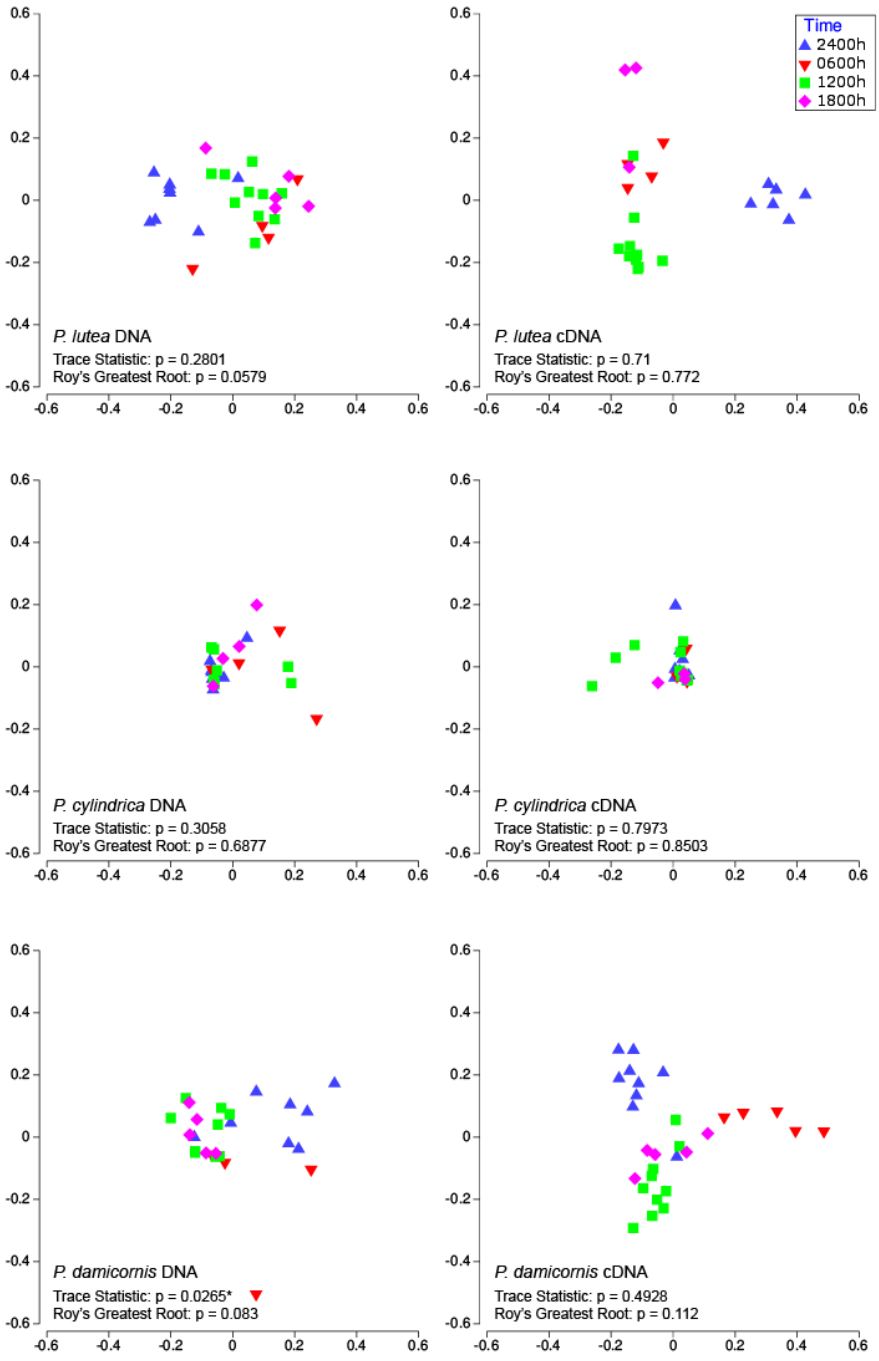
APPENDIX D

Pairwise time comparisons for *Pocillopora damicornis* from the DNA dataset at the microbial species level, based on square root transformed Bray Curtis dissimilarity. Significant differences (*p-value < .05) are in bold. All 2400h and 1200h time points were pooled for this analysis.

TIME COMPARISON		PERMANOVA P-VALUE	PERMDISP P-VALUE
2400h	0600h	0.31	0.31
2400h	1200h	**0.007***	**0.024***
2400h	1800h	0.09	0.30
0600h	1200h	0.072	**0.0011***
0600h	1800h	0.28	0.18
1200h	1800h	0.87	0.83

APPENDIX E

E.1. Significant separation between time points is obtainable for 16S rRNA genes (DNA) for *Pocillopora damicornis*. Canonical Analysis of Principal Coordinates (CAP) of DNA (left) and cDNA (right) based upon square root transformed Bray Curtis dissimilarities at the microbial species level for three coral species, *Porites lutea*, *Porites cylindrica*, and *Pocillopora damicornis*. Timepoints are only significantly different for *P. damicornis* in the DNA dataset (see Appendix E.2). CAP detected no significant differences at the microbial SV level. All 2400h and 1200h time points were pooled for this analysis.



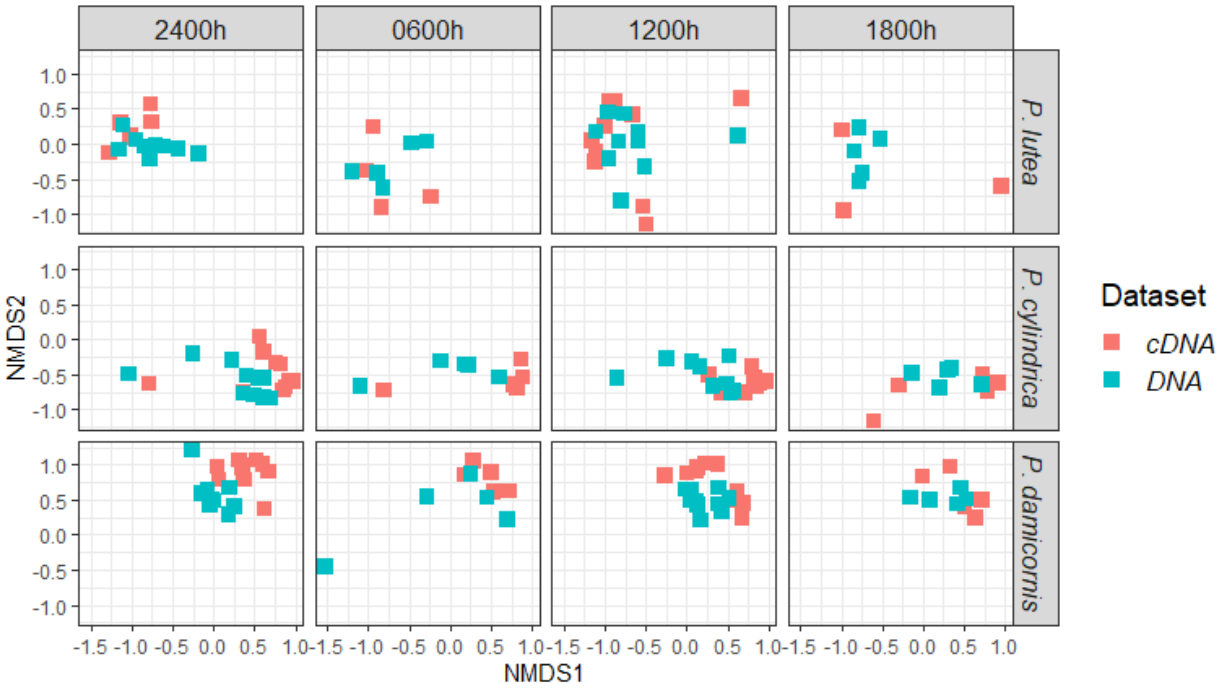
E.2. Cross validation results for the CAP analysis (m=5) of *P. damicornis* from the DNA dataset (see Appendix E.1). All 2400h and 1200h time points were pooled for this analysis.

	Classification				
Original group	**2400h**	**0600h**	**1200h**	**1800h**	**Percent correct**
2400h	6	0	1	1	75
0600h	1	1	2	0	25
1200h	0	0	4	5	44.444
1800h	0	0	4	1	20

Total correct: 12/26 (46.154%)

APPENDIX F

16S rRNA genes (DNA) and transcripts (cDNA) cluster differently at different time points and between different species. Non-metric multidimensional scaling plot of DNA and cDNA datasets separated by coral species (*Porites lutea*, *Porites cylindrica*, and *Pocillopora damicornis*) and time (all 2400h and 1200h time points were pooled for this analysis). nMDS are based upon square root transformed Bray Curtis dissimilarities.



APPENDIX G

Differences between DNA and cDNA for each coral species based on square root transformed Bray Curtis dissimilarities at both the microbial SV (SV) and microbial species (Species) level. Significant differences (*p-value < .05) are in bold.

	Porites lutea	*Porites cylindrica*	*Pocillopora damicornis*
PERMDISP (SV)	0.11	0.21	**0.012***
PERMANOVA (SV)	**0.0001***	**0.0016***	**0.0001***
PERMDISP (Species)	0.085	0.26	**0.008***
PERMANOVA (Species)	**0.0001***	**0.001***	**0.0001***

APPENDIX H

H.1. Taxonomic assignment and average relative abundance of DNA SVs present in all samples of a single coral head. Some SVs were assigned to the same taxonomy and are repeated in the table

Taxonomy	Average Relative Abundance
Porites lutea	
Coral Head 1	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	24.2 ± 10.1
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	4.8 ± 2.8
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium;	2.5 ± 1.1
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;Vibrio cholerae	2.5 ± 1.1
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas;	2.4 ± 0.7
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Midichloriaceae;MD3-55;	1.7 ± 0.6
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;	1.5 ± 0.8
Bacteria;Proteobacteria;;;;;	1.4 ± 0.7
Bacteria;Bacteroidetes;Bacteroidia;Cytophagales;Spirosomaceae;Emticicia;	1.4 ± 0.6
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Midichloriaceae;MD3-55;uncultured bacterium	1.3 ± 0.4
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;	1.2 ± 0.9
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;Cryomorphaceae;uncultured;	0.7 ± 0.2
Bacteria;Proteobacteria;Gammaproteobacteria;Francisellales;Francisellaceae;[Caedibacter] taeniospiralis group;uncultured bacterium	0.4 ± 0.1
Coral Head 2	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	6.9 ± 3.4
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas;	4.8 ± 1.6
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;Vibrio cholerae	4.5 ± 1.6
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium;	4.0 ± 1.3
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;	3.3 ± 1.1
Bacteria;Proteobacteria;Alphaproteobacteria;Reyranellales;Reyranellaceae;Reyranella;	2.3 ± 0.8
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Flavobacterium;	1.4 ± 0.7
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;Crocinitomicaceae;Fluviicola;Fluviicola hefeinensis	0.9 ± 0.4
Coral Head 3	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus;	33.0 ± 11.8
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	3.0 ± 1.3
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium;	1.4 ± 0.7
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Agaribacter;uncultured bacterium	1.2 ± 0.5
Coral Head 4	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	5.5 ± 2.4
Coral Head 5	
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;Vibrio cholerae	5.0 ± 2.8
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas;	4.4 ± 2.2
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	4.0 ± 1.0
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium;	2.8 ± 1.8
Bacteria;Bacteroidetes;Bacteroidia;Cytophagales;Spirosomaceae;Emticicia;	1.8 ± 1.3
Porites cylindrica	
Coral Head 6	
Bacteria;Proteobacteria;Gammaproteobacteria; Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	95.3 ± 1.8

Coral Head 7	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	94.3 ± 3.1
Bacteria;;;;;;	0.3 ± 0.2
Coral Head 8	
Bacteria;Proteobacteria;Gammaproteobacteria; Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	95.0 ± 1.3
Coral Head 9	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	95.8 ± 1.4
Bacteria;Bacteroidetes;Bacteroidia;;;;	0.1 ± 0.05
Coral Head 10	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	54.2 ± 13.4
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	1.0 ± 0.4
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	0.5 ± 0.1
Pocillopora damicornis	
Coral Head 11	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	81.6 ± 3.2
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	5.9 ± 1.3
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	1.6 ± 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	0.9 ± 0.5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	0.4 ± 0.1
Coral Head 12	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	63.5 ± 8.1
Bacteria;;;;;;	5.1 ± 1.0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	4.6 ± 0.9
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;Vibrio cholerae	2.5 ± 1.3
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	2.5 ± 0.5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	1.2 ± 0.2
Bacteria;Bacteroidetes;Bacteroidia;Cytophagales;Amoebophilaceae;Candidatus Amoebophilus;uncultured Sphingobacteriales bacterium	0.9 ± 0.3
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera;uncultured Planctomycetales bacterium	0.8 ± 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	0.7 ± 0.3
Coral Head 13	
No SVs Present in all time points	N/A
Coral Head 14	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	55.8 ± 15.5
Bacteria;;;;;;	3.1 ± 1.2
Coral Head 15	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	79.6 ± 4.8

Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	5.6 ± 0.7
Bacteria;;;;;;	2.3 ± 0.8
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	1.5 ± 0.4

H.2. Taxonomic assignment and average relative abundance of cDNA SVs present in all samples of a single coral head. Some SVs were assigned to the same taxonomy and are repeated in the table

Taxonomy	**Average Relative Abundance**
Porites lutea	
Coral Head 1	
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Midichloriaceae;MD3-55;	9.5 ± 3.3
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Midichloriaceae;MD3-55;uncultured bacterium	4.7 ± 0.3
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	3.0 ± 1.1
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;;;	2.4 ± 1.1
Bacteria;Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Neisseriaceae;Neisseria;	2.1 ± 1.2
Bacteria;Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;;	1.6 ± 0.5
Bacteria;Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;;	1.1 ± 0.3
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Porphyromonas;	0.7 ± 0.4
Bacteria;Proteobacteria;Gammaproteobacteria; Oceanospirillales;Endozoicomonadaceae;Endozoicomonas;uncultured bacterium	0.7 ± 0.2
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;Flavobacteriaceae;NS5 marine group;uncultured Flavobacteriaceae bacterium	0.7 ± 0.3
Bacteria;Proteobacteria;Gammaproteobacteria;Francisellales;Francisellaceae;[Caedibacter] taeniospiralis group;uncultured bacterium	0.7 ± 0.2
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;NS9 marine group;;	0.6 ± 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;HOC36; uncultured sediment bacterium;uncultured sediment bacterium;uncultured sediment bacterium	0.6 ± 0.3
Coral Head 2	
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	5.2 ± 1.2
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Midichloriaceae;MD3-55;	3.4 ± 2.3
Bacteria;Proteobacteria;;;;;	2.4 ± 2.1
Bacteria;Proteobacteria;Gammaproteobacteria;Francisellales;Francisellaceae;[Caedibacter] taeniospiralis group;uncultured bacterium	2.0 ± 1.8
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;	1.6 ± 0.9
Bacteria;Proteobacteria;Gammaproteobacteria; Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas;	1.5 ± 1.2
Bacteria;Proteobacteria;Gammaproteobacteria; Betaproteobacteriales;Neisseriaceae;Neisseria;	1.0 ± 0.8
Bacteria;Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae;Synechococcus CC9902;	0.9 ± 0.7
Bacteria;Firmicutes;Bacilli;Lactobacillales;Aerococcaceae;Abiotrophia;uncultured bacterium	0.5 ± 0.4
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Porphyromonas;	0.3 ± 0.1
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;;	0.2 ± 0.1
Coral Head 3	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus;	34.2 ± 9.3
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.6 ± 0.2
Coral Head 4	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	4.0 ± 3.2
Coral Head 5	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	25.5 ± 24.2
Porites cylindrica	
Coral Head 6	

Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	79.0 ± 15.7
Coral Head 7	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	98.9 ± 0.5
Coral Head 8	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	94.7 ± 2.9
Bacteria;Bacteroidetes;Bacteroidia;;;;	1.6 ± 0.6
Coral Head 9	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	98.1 ± 0.8
Coral Head 10	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	70.7 ± 14.0
Pocillopora damicornis	
Coral Head 11	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	85.0 ± 3.7
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	6.1 ± 1.2
Bacteria;Epsilonbacteraeota;Campylobacteria;Campylobacterales;;;	0.5 ± 0.1
Coral Head 12	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	80.0 ± 3.3
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	6.2 ± 0.7
Bacteria;;;;;;	2.3 ± 0.5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	1.5 ± 0.4
Bacteria;Epsilonbacteraeota;Campylobacteria;Campylobacterales;;;	1.3 ± 0.5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.7 ± 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.7 ± 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.4 ± 0.1
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera;uncultured Planctomycetales bacterium	0.3 ± 0.04
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.2 ± 0.1
Coral Head 13	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	8.9 ± 2.5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	76.1 ± 6.2
Bacteria;;;;;;	1.9 ± 0.4
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	1.8 ± 0.6
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera;uncultured Planctomycetales bacterium	0.4 ± 0.1
Coral Head 14	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	84.8 ± 4.8

Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	6.5 ± 1.0
Bacteria;;;;;;	1.2 ± 0.3
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	0.6 ± 0.1
Coral Head 15	
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	76.0 ± 12.4
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Endozoicomonadaceae;Endozoicomonas; uncultured bacterium	5.9 ± 1.2
Bacteria;;;;;;	0.8 ± 0.4